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ORIGINAL ARTICLE

Serum Biglycan as a Diagnostic Marker for Non-Alcoholic Steatohepatitis and Liver Fibrosis

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SUMMARY

Background: **Non-alcoholic steatohepatitis (NASH) has risen in prevalence substantially through the years. Although course and progression of the disease are variable, fibrosis is the most important factor. We intended to explore utility of serum biglycan (BGN) in NASH and its capacity in anticipating liver fibrosis.**
Methods: **Serum tests of consecutive patients with biopsy-confirmed NASH and age, gender-matched healthy volunteers were utilized to evaluate serum BGN levels using ELISA kits. The correlation between BGN and histopathological highlights of NASH was examined. While patients with fibrosis scores < 2 were assembled in mild and scores of (≥ 2) were in significant fibrosis groups. Univariate/multivariate regression analyses were performed to assess the independent predictive variables of liver fibrosis. Receiver operating characteristics (ROC) were applied to locate the best cutoff values of BGN for NASH and fibrosis.**
Results: **Seventy patients with NASH and 70 controls were recruited in the study. BGN levels were lower in NASH patients contrasted with controls 137.70 ± 33.12 pg/mL vs. 259.61 ± 187.34 pg/mL, respectively, and p < 0.001. In correlation, serum BGN was related to liver fibrosis and inflammation. The comparison between mild and significant fibrosis groups regarding BGN was as follows 155.92 ± 49.97 pg/mL vs. 390.07 ± 214.746 pg/mL, respectively, (p < 0.001). In multivariate analyses, BGN was an independent predictive factor of significant fibrosis (OR, 1.030; 95% CI: 1.011 - 1.048; p < 0.001). ROC analysis revealed that BGN was statistically significant in determination of significant fibrosis (AUROC, 0.955; 95% CI, 0.877 - 0.990; p < 0.001). Best cutoff value was 189.58 pg/mL with the best sensitivity (93.55%) and specificity (87.18%).**
Conclusions: **Serum BGN may be a new non-invasive indicative marker for the presence of NASH, significant fibrosis, and a treatment goal in the disease process.**
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KEY WORDS

biglycan, liver fibrosis, NAFLD, NASH, non-invasive marker

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has substantially risen in prevalence globally through the years, along with the developing epidemic of weight problems [1]. NAFLD is one of the main causes of chronic liver disorder in recent years. Although course and progression of the disease are variable, fibrosis is the most important factor responsible for all and liver associated

mortality [2]. Approximately one-third of western populations have NAFLD and approximately 20% of them are expected to develop non-alcoholic steatohepatitis (NASH) and fibrosis. Also, approximately 20% of patients with NASH eventually will develop cirrhosis [3, 4]. Regardless of fibrosis, the addition of ballooning and lobular or portal inflammation to the steatosis of hepatocytes are diagnostic features for NASH [5]. NASH with fibrosis is the most significant hazard factor which plays a role in the development of cirrhosis and other liver-related morbidity [6]. Liver biopsy is still the widespread gold standard and accurate procedure for the analysis and diagnosis of fibrosis; however, it has many limitations: the biopsy length displays a small part of a liver condition and the invasive nature of the procedure may be complicated with pain, harm to liver parenchyma, and hemorrhage. The liver biopsy cannot distinguish early from significant fibrosis. It does not seem to be very logical to perform a liver biopsy due to the high prevalence of the disorder and its high occurrence especially in the elderly population [7]. Therefore, it is important to find markers that can evaluate the degree of liver fibrosis, noninvasively.

Biglycan (BGN) has a glycanated proteoglycan core protein with the aid of two GAG chains and is part of the superfamily of proteoglycans known as the small leucine repeat proteoglycan (SLRP) family [8]. BGN presents in nearly all of the organs of body, but its dispersion is not equal and mostly radiated in the extracellular matrices (ECM), especially on the cell surface of several featured cells [9-12]. ECM symbolizes a family of macromolecules, covering collagen, non-collagen glycoprotein, glycosaminoglycans, matrix proteins, and proteoglycans. BGN is a released proteoglycan interrelated in collagen fibril installation while its particles seem to be related with collagen remodeling in the course of the pathogenesis of diseases that encompass dysregulated ECM remodeling, just like hepatic fibrosis. BGN has a critical key role in the course of fibrogenesis by arranging cytokine activity such as TGF-β and TNF-α [13]. Our purpose in this study was to look for the clinical efficacy and capability of serum BGN levels in establishing the existence of NASH by comparing with the gender and age-matched healthy volunteers and also to interpret the correlation between serum BGN and liver fibrosis and other histopathological characteristics of NASH.

MATERIALS AND METHODS

Patients

All consecutive patients with biopsy-confirmed NASH and age- and gender-matched healthy volunteers attending the Department of Gastroenterology, Faculty of Medicine, Gazi University, a tertiary reference center (Ankara, Turkey), were included in this cross-sectional research study. Patients over 18 years old with persistently high liver enzymes and steatosis on ultrasonography with no other reasons were appropriate to take part in the study. Other than these, patients with viral hepatitis, sclerosing cholangitis, autoimmune hepatitis, primary biliary cholangitis, α1-antitrypsin deficiency, Wilson's disease, hemochromatosis, drug-induced liver disease, malignancy, poor nutritional status, and/or changed lipid metabolisms were not eligible for this work. Alcohol intake of > 20 g/day for men and > 10 g/day for women and hormone replacement medication therapy or drugs causing steatosis were noted also as exclusion criteria. The exclusions due to these situations were done depending on the evidence-based laboratory, clinical, and histopathological criteria. Patients with DM under control with antidiabetics and/or insulin therapy and patients with HT under control with angiotensin converting enzyme inhibitors were included in the study. The healthy control group consisted of consecutive age- and gender-matched volunteers with the aminotransferase levels in normal range and normal abdominal ultrasonography, without any such situations as alcohol consumption, drug or herbal substance, and liver disease and were negative regarding viral hepatitis serology. All the patients and volunteers were members of the same ethnicity.

Evaluation of clinical and laboratory features

Anthropometric assessment of height in kilograms (kg) and body weight in meters (m) was detected and body mass index (BMI) was computed by dividing the weight in kg with the square of height in m [14]. Before performing the biopsy, venous blood examples were taken from the antecubital vein of patients between 8:00 - 10:00 a.m. after 12 hours of fasting. Complete blood cell count (CBC), including hemoglobin, white blood cells, lymphocytes, neutrophils, and platelets, was computed using a Beckman Coulter Gen-S Programmed Analyzer (Beckman Coulter, High Wycombe, UK) within 2 hours of sampling. Plasma glucose and lipid parameters (total cholesterol, high-density lipoprotein cholesterol, and triglycerides) were evaluated by existing kits. Low-density lipoprotein cholesterol levels were computed by the Friedewald formula [15]. Albumin, alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase, and alkaline phosphatase levels were calculated using a Roche Modular System automatic analyzer (Roche Cobas Integra 800; Roche, Indianapolis, IN, USA) in a hospital laboratory. Demographical, clinics, and laboratory information were recorded in a database by a blinded clinician to completely eliminate bias.

Evaluation of serum BGN levels

Blood samples were centrifuged at 1,600 g for 15 minutes and then kept at -80°C until assessment date. Boster's Human Biglycan enzyme-linked immunosorbent assay (ELISA) kit (Human Biglycan ELISA Kit, Boster Immunoleader, Boster Biological Technology Co., Ltd., Pleasanton, CA, USA) based on a standard sandwich ELISA technology was applied to find human serum

BGN in accordance with instructions of the manufacturer. After the ELISA steps were performed according to the instructions, the optical density (OD) of each example was read spectrophotometrically within 15 minutes using an automated ELISA reader which was set to 450 nanometer wavelength. Serum BGN levels were computed using OD values of standards with common concentrations using regression-correlation analysis by CurveExpert Basic (Version 1.4; CurveExpert, Hixson, TN, USA) statistical software package. The test has good sensitivity and high specificity in the assessment of BGN. The assay interval was between 156 pg/mL and 10,000 pg/mL. The specificity of the test was natural and recombinant human BGN. The sensitivity of the assay kit was lower than 10 pg/mL. There was no detectable cross-reactivity with other proteins. The coefficient of variation (CV) concerning intra-assay precision was 3.7 - 4.9% and 4.1 - 5.2% for the inter-assay precision.

Liver biopsy and histopathologic assessment

Percutaneous liver biopsy was applied by a skilled clinician using a 16-gauge Hepafix (Braun Melsungen, Melsungen, Germany) single-use needle. All liver biopsy samples covering more than 12 whole portal tracts and longer than 20 - 25 mm were taken into consideration for the study. Liver tissue samples were examined by the same professional hepatopathologist blinded to the data of patients. Hematoxylin and eosin (HE) and Masson trichrome stains were utilized in histopathological assessment of formalin-fixed and paraffin-embedded samples.

The diagnosis of NAFLD was assessed based on Brunt's criteria. Histological features were staged according to the NAFLD scoring system proposed by the National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network. Hepatic steatosis was graded from 1, 2, or 3 according to the steatosis ratio as 5 to < 33%, 33 - 66% and > 66%, respectively. Lobular inflammation was described as the total evaluation of all inflammation; no foci as score 0, less than 2 foci per x 200 field as score 1, 2 - 4 foci per x 200 field as score 2, more than 4 foci per x 200 field as score 3. Ballooning scoring was termed as: score 0 if no ballooning of hepatocytes, score 1 if there is occasional and score 2 if there is obvious ballooning. Histopathologically, the total NAFLD activity score (NAS) was calculated as a sum of steatosis (1 - 3), lobular inflammation (0 - 3), and ballooning (0 - 2). Fibrosis was staged as follows: stage 0, no liver fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis, and stage 4, cirrhosis [16,17]. Fibrosis can be categorized as mild (stage F0-1) and significant fibrosis groups (stage F2-4), the prognosis of which are different from each other [18]. The pathologic protocols regarding liver fibrosis lack data assessing interobserver variability and their inadequate ability to guess mortality related fibrosis. The liver biopsy cannot distinctly project the fibrotic variation existing in the whole liver. As liver fibrosis procedures are not homogenous, samples from different areas may detect various grades. In early grades of liver fibrosis, liver function tests may be in normal range and patients may have no complaints. Therefore, estimating fibrosis by a non-invasive method is seen as a seriously important area.

Ethics

This study was designed and fulfilled the criteria in accordance with the 1975 Helsinki Declaration, which was renewed in 2008, and was confirmed by the local ethics committee. All of the individuals gave written and signed informed consent before joining the study.

Statistical analysis

Statistical analyses were done by using SPSS (v23; IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA) and MedCalc (version 14; MedCalc, Mariakerke, Belgium). The normality of factors was controlled by using visible histograms, analytical approaches, and probability plots (Kolmogorov-Smirnov/Shapiro-Wilk's test) where appropriate. Descriptive results for non-normally distributed parameters and ordinal parameters were represented as median and standard error or interquartile range. For the normally distributed parameters the results were shown as mean and standard deviation (SD). Student's *t*-test was used for normally distributed parameters, though for non-normally ones, the Mann-Whitney U-test was used in comparing two groups. Regarding correlation analysis, Spearman's correlation coefficients (rs) were computed to investigate the correlation between serum BGN levels and liver histopathological features such as inflammation, ballooning, steatosis, and fibrosis and the clinical properties of patients with biopsy-confirmed NASH. A univariate analysis to determine the variables determining significant liver fibrosis was carried out using the χ^2, Fisher's exact, Student's *t*-test, and Mann-Whitney U-tests. In the multivariate logistic regression evaluation, the feasible variables found significant in univariate analyses were then assessed to perceive independent predictors in significant liver fibrosis. Model suitability was assessed by using Hosmer-Lemeshow goodness-of-fit statistics. The predicting ability of serum BGN in the presence NASH and significant liver fibrosis was investigated by receiver operating characteristics (ROCs) analysis. The significant level with the best sensitivity and specificity in the area under the curve (AUC) analysis was detected as the optimal cutoff value. When the significant cutoff value was obtained the sensitivity and specificity were calculated. We also used the 1,000 bootstrap samples method for choosing the final method. In every bootstrap generation, we refitted the model and assessed the performance in the "all bootstrap samples" (obvious overall performance) and in our original data. Performance was presented in terms of area under receiver operating characteristic (AUROC). A 5% type 1 error was used to show statistical significance.

RESULTS

Seventy consecutive patients with biopsy-confirmed NASH and 70 age- and gender-matched healthy volunteers who fulfilled the inclusion and exclusion criteria were recruited to the study. The median ages of healthy controls and patients were 50 (24 - 76) and 50 (18 - 72), respectively, and 61.4% of both groups were female ($p > 0.05$). The mean BMI of healthy individuals was lower than that of NASH patients (24.90 ± 2.54 vs. 29.55 ± 4.58), respectively, and there was a statistically significant difference ($p < 0.01$). Fasting glucose levels of healthy controls were also lower than those of the patients (88.69 ± 13.75 vs. 109.26 ± 38.54), respectively ($p < 0.001$). The platelet counts of healthy individuals and patient groups were as follow 258 ± 7.2 vs. 243 ± 10.85, respectively, and the difference was statistically significant ($p < 0.025$). When comparing serum BGN levels between healthy individuals and patients, the mean ± SD of the values were 137.70 pg/mL ± 33.12 vs. 259.61 ± 187.34 pg/mL, respectively, and the comparison was statistically significant ($p < 0.001$). The clinical and demographical features of healthy individuals and patients with NASH are summarized in Table 1. The fibrosis grades in the study were F0 in 10 (14.3%), F1 in 29 (41.4%), F2 in 11 (7.7%), F3 in 13 (9.2%), and F4 among 7 (4.9%) patients. There were 39 patients (55.7%) in the mild fibrosis group (F0-1) and 31 patients in the significant fibrosis group (F2-4) (44.3%). When we grouped NASH patients as mild and significant groups and compared serum BGN levels 155.92 ± 49.97 pg/mL vs. 390.07 ± 214.746 pg/mL, respectively, we found a statistically significant difference ($p < 0.001$). We also investigated the comparison between mild-moderate and advanced fibrosis groups (fibrosis scores 0 - 2 vs. 3 - 4) and BGN levels of 171.54 ± 56.32 pg/mL vs. 479.80 ± 218.70 pg/mL, respectively, and $p < 0.001$.

Correlation between serum biglycan and clinical/histopathological characteristics of patients with NASH

The correlations between serum BGN levels and clinical and pathological features of patients with biopsy-confirmed NASH were investigated using Spearman's correlation analysis with the 1,000 bootstrapping method. A statistically significant negative correlation was found with platelet count (rs = -0.48; 95% confidence interval (CI): -0.695 to -0.22; $p < 0.001$). There was positive correlation with international normalized ratio (INR) as (rs = 0.48; 95% CI: 0.283 - 0.652); $p < 0.001$. Also, there was statistically significant correlation with inflammation (rs = 0.47; 95% CI: 0.254 - 0.653; $p = 0.001$); NAS (rs = 305; 95% CI: 0.07 - 0.52; $p = 0.012$); and a highly significant positive correlation with fibrosis (rs = 0.890; 95% CI: 0.805 - 0.948; $p < 0.001$). The correlation of serum BGN levels with the clinical and histopathological characteristics of patients with biopsy proven NASH is presented in Table 2.

Univariate/multivariate logistic regression analysis for identifying significant fibrosis

Univariate analysis in identification of significant fibrosis determined that age (odds ratio (OR): 1.056, 95% CI: 1.011 - 1.114, $p = 0.045$); platelet counts: (OR: 0.984; 95% CI: 0.975 - 0.993; $p = 0.001$); albumin: (OR: 0.154; 95% CI: 0.035 - 0.683; $p = 0.014$); AST: (OR: 1.036, 95% CI: 1.011 - 1.062, $p = 0.004$); INR: (OR: 947.25; 95% CI: 2.83 - 3,156.4; $p < 0.001$); and serum BGN (OR: 1.031; 95% CI: 1.016 - 1.047; $p < 0.001$) were statistically significant independent predicting factors. The factors that were statistically significant in the univariate logistic regression model were additionally brought into a multivariate logistic regression analysis and serum BGN level kept on being statistically significant and an independent predictor of the existence of significant fibrosis due of NASH: (OR: 1.030; 95% CI: 1.011 - 1.048 - 0.949; $p < 0.001$). Univariate and multivariate analyses of variables associated with significant fibrosis in patients with biopsy-confirmed NASH using logistic regression models are shown in Table 3.

Predicting the ability of BGN in the presence of NASH and significant fibrosis

The diagnostic abilities of serum BGN in predicting the existence of NASH and significant liver fibrosis were assessed by AUROC. The AUROC analysis recommended that a cutoff level of 193.141 pg/mL had best sensitivity (47.14%) and specificity (98.57%) in identifying patients with NASH, with an AUROC of 0.768 (95% CI: 0.689 - 0.835), and was statistically significant ($p < 0.001$). In the 1,000 bootstrapping model the result continued to be statistically significant with a 95% CI: 0.329 - 0.557, Standard Error (SE): 0.0397 between the levels of > 192.56 to < 203.878, ($p < 0.001$). The AUROC curve of serum BGN for the differentiation of significant fibrosis from mild fibrosis in NASH was statistically significant (AUROC, 0.955; 95% CI: 0.877 - 0.990; $p < 0.001$), with the best cutoff level of 189.58 pg/mL and the best sensitivity (93.55%) and specificity (87.18%), $p < 0.001$. In 1,000 bootstrapping model the result was also statistically significant (95% CI: 0.64 - 0.88, SE: 0.0235) between the levels of > 157.908 to < 222.947 and $p < 0.001$.

DISCUSSION

In the current research, we presumed that patients with biopsy-affirmed NASH had elevated serum BGN levels as opposed to age- and gender-matched healthy volunteers. Ordinarily, the elevation in BGN is associated with the increase in fibrosis progression as individuals with significant liver fibrosis had higher serum BGN contrasted with the mild fibrosis group. A positive powerful correlation was seen between BGN levels and fibrosis scores. Also, on the basis of univariate/multivariate logistic regression analysis, serum BGN was de-

Table 1. The clinical and demographical features of healthy individuals and patients with NASH are summarized.

Factors	Healthy control (n = 70)	NASH group (n = 70)	p-value
Age, years	50 (24 - 76)	50 (18 - 72)	0.56
Gender, female, %	43, 61.4%	43 61.4%	1.0
BMI (kg/m^2)	24.90 ± 2.54	29.55 ± 4.58	< 0.001
Fasting glucose (mg/dL)	88.69 ± 13.75	109.26 ± 38.54	< 0.001
ALT (IU/mL)	20.96 ± 7.63	68.53 ± 59.80	< 0.001
AST (IU/mL)	20.57 ± 5.61	52.93± 54.69	< 0.001
ALP (IU/mL)	71.46 ± 21.08	107.46 ± 65.04	< 0.001
GGT (IU/mL)	22.57 ± 9.88	68.63 ± 46.97	< 0.001
Hemoglobin [a], g/dL	13.7 ± 0.14	13.94 ± 0.23	0.122
Platelet count [a], $/mm^3 \times 10^3$	258 ± 7.2	243 ± 10.85	0.025
WBC	8,314 ± 2,247.02	7,451 ± 2,161.50	0.046
Albumin, mg/dL	4.28 ± 0.41	4.32 ± 0.40	0.136
Total cholesterol, mg/dL	209.97 ± 39.95	202.95 ± 43.59	0.350
HDL, mg/dL	50.10 ± 11.01	43.15 ± 9.85	0.002
LDL, mg/dL	132.92 ± 34.65	125.01 ± 34.20	0.244
TG, mg/dL	131.64 ± 55.52	174.03 ± 77.74	0.001
VLDL, mg/dL	25.80 ± 11.59	33.77 ± 15.68	0.009
BGN pg/mL	137.70 ± 33.12	259.61 ± 187.34	< 0.001

ALP - alkaline phosphatase, ALT - alanine aminotransferase, AST - aspartate amino transferase, BGN - biglycan, BMI - body mass index, GGT - gamma glutamyl transferase, HDL - high density lipoprotein, LDL - low density lipoprotein, NAFLD - non-alcoholic fatty liver disease, TG - triglyceride, WBC - white blood cells. Normally distributed variables are represented as mean ± standard deviation (SD).
[a] Non-normally distributed factors are represented as median ± standard error (SE).

Table 2. The correlation of serum BGN levels with the clinical and histopathological characteristics of patients with biopsy proven NASH.

Factors	Spearman's correlation coefficients (r_s)	95% CI [a]	p-value
Clinical features			
ALT	0.198	0.081 - 0.033	0.019
AST	0.256	0.078 - 0.095	0.002
ALP	0.076	-0.074 - 0.232	0.371
GGT	0.255	0.110 - 0.401	0.002
BMI	0.104	-0.106 - 0.312	0.302
INR	0.48	0.283 - 0.652	< 0.001
Albumin level	-0.250	-0.434 - 0.068	0.012
Platelet count	-0.484	-0.695 - -0.219	< 0.001
Total bilirubin	0.012	-0.194 - 0.227	0.907
Glucose	0.217	0.013 - 0.417	0.030
Total cholesterol	-0.135	-0.352 - 0.088	0.180
HDL	0.088	-0.103 - 0.281	0.386
LDL	-0.079	-0.293 - 0.138	0.437
Triglyceride	-0.239	-0.424 - -0.054	0.017
Histopathological features			
Steatosis	-0.045	-0.285 - 0.191	0.801
Inflammation	0.468	0.254 - 0.653	0.001
Ballooning	0.192	-0.058 - 0.407	0.117
NAS	0.305	0.07 - 0.52	0.012
Fibrosis	0.890	0.805 - 0.948	< 0.001

ALT - alanine aminotransferase, ALP - alkaline phosphatase, AST - aspartate amino transferase, BMI - body mass index, CI - confidence interval, GGT - gamma glutamyl transferase, HDL - high density lipoprotein, INR - international normalized ratio, LDL - low density lipoprotein, NAFLD - non-alcoholic fatty liver disease, NAS - non-alcoholic fatty liver disease activity score; r_s - Spearman's correlation coefficient.
*** [a] With the 1,000 bootstrapping method.**

Table 3. Univariate and multivariate analyses of variables associated with significant fibrosis in patients with biopsy-confirmed NASH by logistic regression models.

Factors	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
Age	1.056	1.001 - 1.114	0.045	1.101	0.935 - 1.298	0.249
Gender	1.011	0.384 - 2.662	0.983			-
Albumin	0.154	0.035 - 0.683	0.014	0.248	0.015 - 3.977	0.325
Platelet count	0.984	0.975 - 0.993	0.001	1.002	0.983 - 1.020	0.863
BMI	1.066	0.959 - 1.185	0.236			-
Hemoglobin	0.925	0.677 - 1.265	0.625			-
WBC	1.0	1.0 - 1.0	0.174			-
ALT	1.009	0.999 - 1.019	0.067			-
AST	1.036	1.011 - 1.062	0.004	1.072	1.014 - 1.134	0.014
ALP	1.007	0.998 - 1.017	0.142			-
GGT	1.009	1.009 - 1.020	0.089	1.015	0.996 - 1.036	0.128
Total protein	2.041	0.540 - 7.712	0.293			-
Fasting glucose	1.007	0.995 - 1.019	0.271			-
INR	947.25	2.83 - 3,156.4	0.001	3,212.95	0.034 - 305E + 10	0.139
Total cholesterol	1.003	0.991 - 1.014	0.636			-
HDL	1.024	0.972 - 1.080	0.370			-
LDL	1.006	0.991 - 1.021	0.453			-
TG	0.996	0.989 - 1.002	0.204			-
BGN	1.031	1.016 - 1.047	< 0.001	1.030	1.011 - 1.048	< 0.001

ALT - alanine aminotransferase, ALP - alkaline phosphatase, AST - aspartate amino transferase, BGN - biglycan, BMI - body mass index, CI - confidence interval, GGT - gamma glutamyl transferase, HDL - high density lipoprotein, INR - international normalized ratio, LDL - low density lipoprotein, NASH - non-alcoholic steatohepatitis, OR - odds ratio, TG - triglyceride, WBC - white blood cells.

fined as an independent predictive factor for the existence of significant liver fibrosis. As the progression of liver fibrosis in NASH is dynamic, which may result in end-stage liver disease, the search for a non-invasive diagnostic marker identifying the histopathological features and fibrosis of NASH is essential to prevent the complications that may occur due to liver biopsy. The present study showed that serum BGN level is a new non-invasive marker in NASH and significant liver fibrosis.

We found a good positive relationship among BGN and liver fibrosis stages and, furthermore, a correlation with inflammation; however, no relationship was found with other histopathological features, for example, ballooning and steatosis. The BGN levels were elevated in significant fibrosis and a more noteworthy increase was seen in advanced fibrosis. Although 1,000 sample bootstrapping models were applied in univariate and multivariate logistic regression analysis, they showed that BGN was an independent predictive variable in the discovery of significant hepatic fibrosis. When these results were taken into consideration, biglycan was simply associated with liver fibrosis and its progression. As we did not analyze straightforward steatosis and NASH, there was no correlation with histopathologically proven steatosis.

Liver fibrosis is found in the area where injury is most serious especially in chronic liver diseases. Extracellular matrix proteins covering a group of macromolecules like collagen, non-collagen glycoprotein, matrix proteins, glycosaminoglycans, and proteoglycans play a role in fibrotic disorders [19]. BGN is a released proteoglycan, participating in collagen fibril gathering, and furthermore, its fragmentation is by all accounts related with collagen turnover during the pathogenesis of disorders including dysregulated ECM renovating such as liver fibrosis [20,21]. With this well-designed study, we have detected that BGN levels are associated with fibrosis and indirectly proved those molecular pathways regarding liver fibrosis. To the best of our knowledge, our study is the first in the literature showing the relationship between BGN and liver fibrosis due to NASH. Genovese et al. created a liver fibrosis model in rats which showed that BGN cleaved by matrix metallopro-

teinases played a role in pathological ECM remodeling and also detected a significant association between the degree of liver fibrosis and serum BGN levels [22]. In accordance with this study, we have also proven in our study that there is a relationship between serum BGN and NASH-related liver fibrosis. Serum BGN values were higher in patients with NASH than in the healthy individuals, and the highest values were found in patients with significant fibrosis. Serum BGN level may be considered as a treatment goal for NASH and liver fibrosis progression. We think this is a turning point for future studies in the treatment of NASH. This discourse must be proven by major randomized clinical trials. In another study, BGN was stained in fibrotic liver samples of patients with chronic active hepatitis and in normal tissues, and high BGN immunoreactivity was detected in fibrotic areas while in normal liver tissue the staining was frankly in space of Disse [23]. We have also revealed the relationship between BGN and liver fibrosis, which was demonstrated by this hepatic expression research study, non-invasively. Therefore, in our study, a significant distance has been covered in proving the role of BGN in liver fibrosis.

Increasing evidence about the claim that BGN-dependent recruitment of inflammatory cells is based upon a selective utilization of TLRs. By the overexpression of soluble BGN in MyD88, TRIF, and double knock out mice, it was demonstrated that BGN-mediated infiltration of neutrophils occurs exclusively via MyD88 adapter molecule [24]. It is concluded in some studies that BGN-mediated inflammation is a characteristic factor related with many chronic inflammatory disorders like fibrosis [25,26]. New findings show that, other than TLRs, biglycan straightforwardly connects with the cluster of differentiation (CD)14 coreceptor or CD44 receptor so as to drive two unmistakable separate pathways, inflammation and autophagy. Along these lines, it turns out to be increasingly apparent that BGN goes about as an ECM-determined alterative managing inflammatory signaling toward chronic inflammation [27, 28]. In our present well organized study, we scientifically proved this information in the literature by finding that serum BGN level is related to both inflammation and liver fibrosis. This result is very important in clarifying the pathogenesis of NASH related inflammation and liver fibrosis.

In an animal study performed on 52 rats, a hepatic fibrosis model was set up by regulating CCL4. Biomarkers including type 1 collagen, type 3 collagen, type 4 collagen, type 6 collagen, citrulline, vimentin, PCP5, PN3P, P4NP-7S, and biglycan appeared to connect with the level of hepatic fibrosis prompted by CCL4 [29]. The results of our study were compatible with this study and we have confirmed the results of this study.

Our present study has some limitations. First, assessment of liver immunoreactivity of BGN among patients was not done, as combining the results of serum levels and immunoreactivity of BGN would provide more related data on the role of BGN in NASH and progression of liver fibrosis. Second, the study included a relatively small number of participants of the same ethnicity and non-uniform fibrosis groups. We tried to overcome these obstacles by performing univariate/multivariate logistics regression analysis and ROC analysis also with 1,000 bootstrap methods to prove repeatability of the data and found strong association with combining all statistical analysis. However, studies on large population-based cohorts of various ethnicities may give additional information on the role of serum BGN in NASH with liver fibrosis. Third, although there was no biopsy-proven simple steatosis group, we investigated the relationship between BGN and histopathological steatosis and found no correlation, but comparison of BGN in healthy volunteers, simple steatosis and NASH group may be more informative in the evaluation of grading models. Fourth, the storage time of serum samples was different. Long-term, different storage time or occurrence of any repeated freeze-thaw cycles of samples may affect the results but there are no reported data about this topic and this should be resolved by prospective clinical trials [30]. Finally, our analysis was based on one assessment of serum BGN, which may not be able to give information about its value over time. Serial measurements should be assessed to define the role of BGN in the pathogenesis of NAFLD.

CONCLUSION

In conclusion, serum BGN may be a new non-invasive indicative marker for the presence of NASH, significant fibrosis, and a treatment goal in the disease process.

Declaration of Interest:
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